Detection of saxitoxin-producing cyanobacteria in subtropical Brazilian drinking water supply reservoir by quantitative PCR

De Almeida Bispo Moraes, Munique; Rodrigues, R. A. M.; Podduturi, Raju; Jørgensen, Niels O. G.; Calijuri, Maria do Carmo

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DETECTION OF SAXITOXIN-PRODUCING CYANOBACTERIA IN A SUBTROPICAL BRAZILIAN DRINKING WATER SUPPLY RESERVOIR BY QUANTITATIVE PCR

M.A.B. Moraes[1], R.A.M. Rodrigues[1], R. Podduturi[2], N.O.G. Jørgensen[2], M.C. Calijuri[1]

[1] Department of Hydraulics and Sanitation, São Carlos School of Engineering, University of São Paulo, Trabalhador São Carlense 400, 13566-590, São Carlos, Brazil

[2] Department of Plant and Environmental Sciences, University of Copenhagen, Thorvaldsensvej 40, 1871 Frederiksberg, Denmark

Corresponding author: muniquemoraes@usp.br

INTRODUCTION

Toxic cyanobacteria in public water supply reservoirs represent a serious health risk since they can release potent cyanotoxins into the water. Among the cyanotoxins are saxitoxin (STX) and its homologs that form a group of potent neurotoxins called paralytic shellfish toxins. Few studies have focused on occurrence of saxitoxin and the genes involved in the STX biosynthesis in oligotrophic freshwaters.

OBJECTIVE

The aim of this study was to infer the potential toxicity of water from the number of cyanobacterial cells carrying the *sxtA* gene (encodes STX synthesis) in a subtropical and oligotrophic reservoir (Itupararanga), which supplies potable water for approximately 800,000 people in São Paulo State (Brazil).

MATERIAL AND METHODS

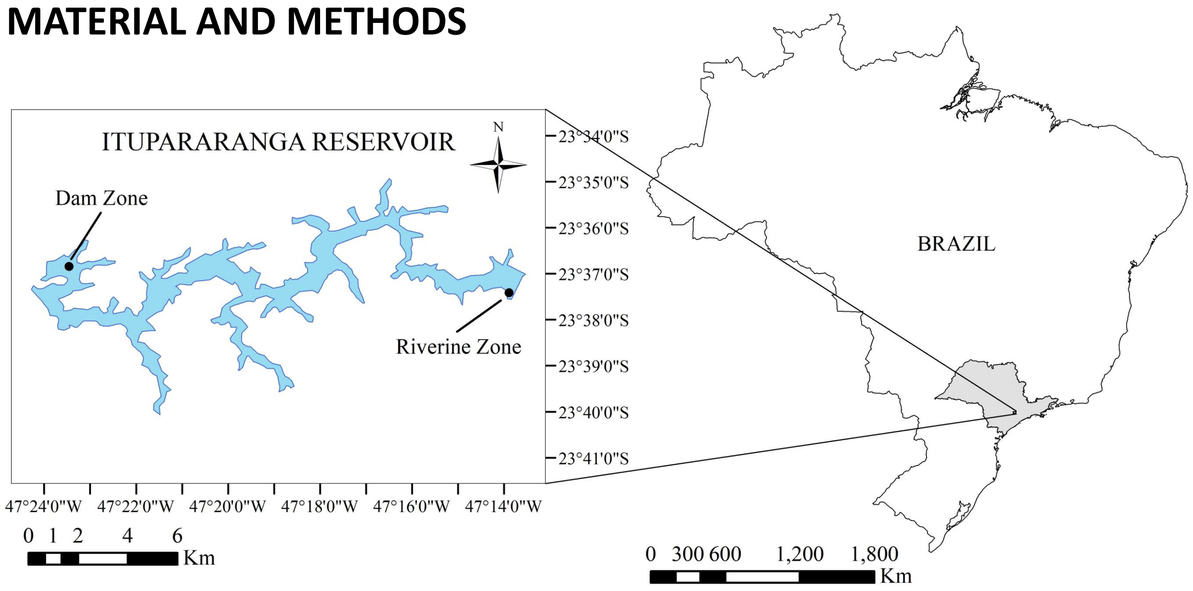

Figure 1. Itupararanga reservoir (São Paulo, Brazil), showing the two sampling stations (dam zone and riverine zone)

- Water samples
  - Nutrients
  - Phytoplankton
  - STX (ELISA)
  - Pigments (HPLC)
  - DNA extraction
- PCR and qPCR
  - Primer set design
    - sxtA-cyano (153 bp PCR product)
  - Standard curve (*Cylindrospermopsis raciborskii* T3)
  - Cyanobacterial strains
  - Environmental samples
  - Sequencing (PCR products)

Statistical analysis Spearman rank order correlations ($p<0.05$)

RESULTS

Table 1. Standard curve parameters and efficiency for the strain *C. raciborskii* T3, when using the *sxtA* target gene

Target gene	Standard	Efficiency (%)	*E*	Slope	R^2
sxtA	*C. raciborskii* T3	95.32	1.95	-3.44	0.99

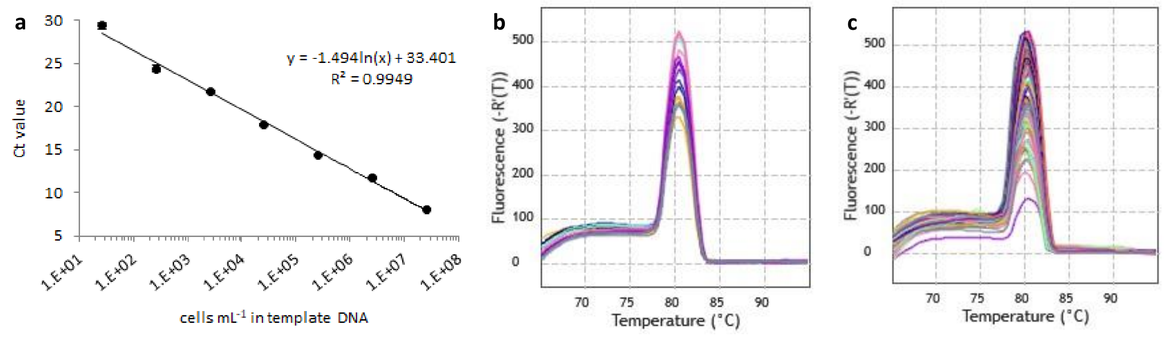

Figure 2. Test of specificity of the primer set. (a) Standard curve of Ct values for tenfold dilutions of known DNA concentration of *C. raciborskii* T3 (in cell equivalents). Error bars provide standard deviations of triplicate amplifications. (b) Melting curves for the *sxtA* primer set for *C. raciborskii* T3 and (c) for environmental samples and standards

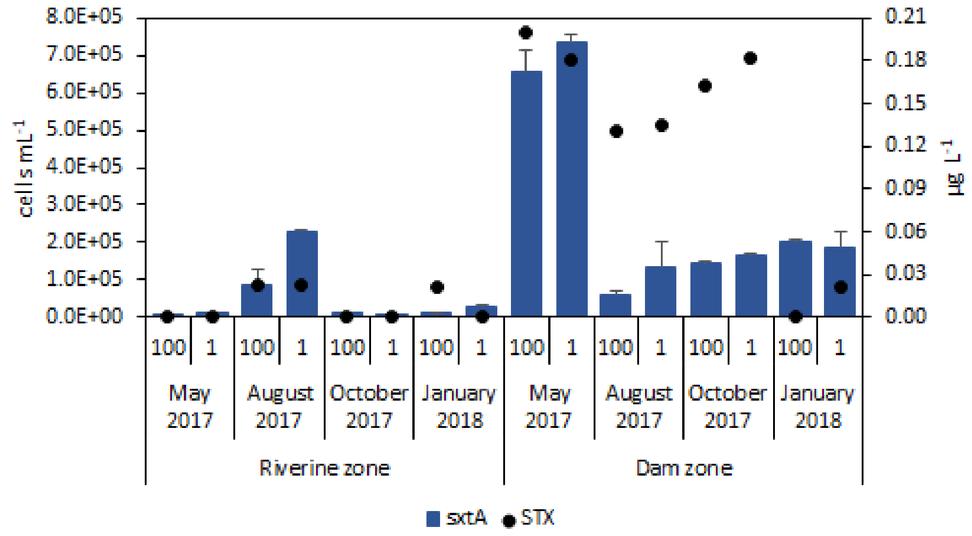

Figure 3. Number of *sxtA* gene (cells mL^{-1}) and saxitoxin concentration (STX, µg L^{-1}) in Itupararanga reservoir. 100: photosynthetically active solar radiation 100%; 1: photosynthetically active solar radiation 1%

Table 2. Spearman rank order correlations for environmental variables in Itupararanga reservoir (rho values, $p<0.05$). Average and standard deviation values are between parentheses

Variables	*sxtA*	STX
Cyanobacteria biomass (3.56±3.30 µg chl *a* L^{-1})	0.73	0.52
Nitrate (0.59±0.26 mg L^{-1})	-0.60	-0.50
Soluble reactive phosphorus (3.08±2.88 µg L^{-1})	-0.61	-0.61
Total phosphorus (31.90±22.33 µg L^{-1})	-0.52	-0.60
TN:TP (89.01±45.92)	0.64	0.82
sxtA (1.68E+05±2.20E+05 cells mL^{-1})	-	0.68
Total STX (0.07±0.08 µg L^{-1})	0.68	-

CONCLUSIONS

The designed primer set sxtA-cyano was efficient for quantification of saxitoxin-encoding gene *sxtA* in environmental samples and allowed the measurement of potentially saxitoxin-producing cyanobacteria in the reservoir. The results obtained also showed the importance of local environmental variables in the overall regulation of saxitoxin production. Therefore, more studies involving quantification of toxic populations are needed to understand the factors responsible for their appearance in particular environmental conditions.

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